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Role of osteocytes in multiple myeloma bone disease

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Abstract

Purpose of review—Despite the increased knowledge of osteocyte biology, the contribution of this most abundant bone cell to the development and progression of multiple myeloma in bone is practically unexplored.

Recent findings—Multiple myeloma bone disease is characterized by exacerbated bone resorption and the presence of osteolytic lesions that do not heal because of a concomitant reduction in bone formation. Osteocytes produce molecules that regulate both bone formation and resorption. Recent findings suggest that the life span of osteocytes is compromised in multiple myeloma patients with bone lesions. In addition, multiple myeloma cells affect the transcriptional profile of osteocytes by upregulating the production of pro-osteoclastogenic cytokines, stimulating osteoclast formation and activity. Further, patients with active multiple myeloma have elevated circulating levels of sclerostin, a potent inhibitor of bone formation which is specifically expressed by osteocytes in bone.

Summary—Understanding the contribution of osteocytes to the mechanisms underlying the skeletal consequences of multiple myeloma bone disease has the potential to provide important new therapeutic strategies that specifically target multiple myeloma–osteocyte interactions.

Keywords

apoptosis; osteoclasts; osteocytes; receptor activator of nuclear factor-kappa B ligand; sclerostin

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Conflicts of interest

The authors have no conflicts of interest to declare.

INTRODUCTION

Multiple myeloma is a plasma cell malignancy characterized by the accumulation of monoclonal plasma cells in the bone marrow and the presence of osteolytic lesions [1]. Multiple myeloma has the highest incidence of bone involvement among malignant diseases. It is estimated that up to 90% of patients with multiple myeloma have evidence of osteolysis in the form of generalized osteopenia or discrete lytic lesions, and up to 60% of multiple myeloma patients develop pathologic fractures [2,3]. Skeletal manifestations, which usually involve the axial skeleton, skull and femur, are the most prominent source of pain and disability in multiple myeloma.

Under physiologic conditions, interactions between cells present in the bone marrow microenvironment result in a balanced and coupled remodeling of bone, a lifelong process responsible for bone damage repair and mineral homeostasis [4]. Bone remodeling is performed by teams of bone-resorbing osteoclasts and bone-forming osteoblasts arranged within structures known as ‘bone remodeling compartments’ (BRCs) [5–7]. In the BRC, bone surface osteoclasts and osteoblasts are physically separated from the bone marrow cavity by a canopy of flat cells (Fig. 1). Functionally, this separation has been proposed to generate a unique microenvironment that facilitates spatial and temporal ‘coupled’ osteoclast resorption and osteoblast formation, minimizing the net change in bone volume during physiological bone remodeling. Although osteoblasts and osteoclast are major participants of the bone remodeling process, osteocytes are the central regulators of bone homeostasis [8]. Osteocytes are the most abundant bone cells, comprising more than 95% of all bone cells, compared with 1–2% osteoclasts and 5% osteoblasts. Despite that their cell bodies lie in lacunae carved within the mineral, osteocytes extensively communicate with each other and with cells on the bone surface and in the marrow through cytoplasmic projections that run within canaliculi [9]. This osteocytic lacunar-canalicular network allows direct cell-to-cell contact between osteocytes and cells near the bone surface and also distributes secreted molecules among all bone and marrow cells as well as into blood vessels to enter the general circulation. Osteocyte-derived molecules regulate the activity of both osteoblasts and osteoclasts by releasing paracrine factors, such as sclerostin (the product of the *Sost* gene) or the receptor activator of nuclear factor-kappa B ligand (RANKL), to locally regulate bone formation and resorption, respectively [10–12]. In addition, emerging evidence supports the notion that osteocytes are responsible for targeting bone remodeling to specific areas in bone [13–15]. In this regard, it has been proposed that apoptotic osteocytes signal to lining cells covering the bone surface, which lift up from bone into the bone marrow and form the canopy that encloses the BRC. Osteoclast precursors are then recruited to the BRC via marrow capillaries in which they mature to osteoclasts and resorb bone, initiating bone remodeling [6].

The bone marrow microenvironment in multiple myeloma is a major contributor to tumor growth and the bone destructive process. Bone remodeling is uncoupled in multiple myeloma and is characterized by generalized osteoclasts activation, which results in exacerbated bone resorption, and by suppressed osteoblast function resulting in a reduction in bone formation [16]. As a result, lytic lesions rarely heal, even when complete remission is attained. Recent evidence demonstrates that the BRC is disrupted in multiple myeloma,

supporting the notion that not only an exchange of soluble factors occurs, but also mechanisms involving direct cell-to-cell contact exist between multiple myeloma cells and bone cells (Fig. 1) [17]. The relevance of these cell-to-cell interactions to the in-vivo bone pathology in multiple myeloma is supported by in-vitro experiments and histological observations that show that extensive bone destruction occurs only in areas of the bones that are highly infiltrated with multiple myeloma cells, whereas bone resorption and formation are balanced in bones with lower tumor burden [18,19]. Interestingly, the biopsies with frequent disruptions in the BRC were also the ones that showed exacerbated resorption without matrix construction [7]. The significance of the cross-talk between multiple myeloma cells and osteoclast and osteoblasts has been a focus of recent investigations. These studies found that multiple myeloma cells stimulate osteoclasts (OC) differentiation and activity through a series of potent osteoclast effectors, such as RANKL, chemokine (C-C motif) ligand 3 (MIP-1), chemokine (C-X-C motif) ligand 12 (SDF-1), interleukin (IL)-1, IL-3, IL-6, vascular endothelial growth factor (VEGF) and tumor necrosis factor, whereas they reduce osteoblast numbers and activity through factors such as IL-7, dickkopf homolog 1 (DKK1), secreted frizzled-related protein (sFRP), VLA-4/vascular cell adhesion molecule 1 (VCAM-1), Fas ligand (FasL), tumor necrosis factor (ligand) superfamily, member 10 (TRAIL) and hepatocyte growth factor (HGF) [20–26]. Although our knowledge of osteocyte function has markedly increased during the last few years, there is a paucity of information about the cross-talk between multiple myeloma and osteocytes and how these interactions contribute to the development and progression of multiple myeloma. In this review, we examine the current findings regarding the role of osteocytes in multiple myeloma bone disease and the potential impact of determining the consequences of osteocyte interactions with myeloma cells that might result in the identification of additional targets for therapeutic development.

OSTEOCYTES AND THE CONTROL OF OSTEOCLAST ACTIVITY

Osteocyte apoptosis is spatially, temporally and functionally linked to bone remodeling partly as a result of the osteocyte cell death [27]. Several studies have shown that regions containing apoptotic osteocytes are targeted for bone resorption, which results in increased bone turnover [14,28,29]. Further, in-vitro experiments demonstrated that osteocyte apoptosis not only can induce recruitment of osteoclast precursors, but also their differentiation [29,30]. Interestingly, increased osteocyte apoptosis has been shown to be induced *in vivo* in areas of microdamage with bone disuse, glucocorticoid administration or estrogen deficiency-induced osteoporosis. Further, exclusive induction of apoptosis of osteocytes was sufficient to increase resorption and bone loss [13]. These findings suggest that osteocytes are involved in pathological conditions involving enhanced bone resorption [14,31,32]. A recent study reported similar findings in multiple myeloma patients [33]. In this study, multiple myeloma patients with bone lesions had fewer viable osteocytes than healthy controls or multiple myeloma patients without bone lesions, partly because of increased osteocyte apoptosis. In line with previous findings in other models, these investigators found a positive correlation between the number of apoptotic osteocytes and the number of osteoclasts in the areas examined. The mechanisms underlying osteoclast recruitment and differentiation induced by osteocyte apoptosis are not fully understood. On

one hand, osteocyte death is commonly accompanied by large increases in RANKL expression, a molecule which not only promotes osteoclast differentiation, but also acts as an osteoclast precursor chemoattractant [34,35]. On the other hand, it is known that multiple myeloma cells affect the transcriptional profiles of osteocytes by upregulating the production of other osteoclastogenic cytokines such as interleukin-11 or MIP1 α , thus increasing their pro-osteoclastogenic properties in an RANKL-independent fashion [33²², 36]. Regardless of the mechanism involved, these results suggest that osteocyte apoptosis might be an early event in multiple myeloma development and progression resulting in enhanced osteoclast migration and production. In this regard, bisphosphonates, the standard of care for multiple myeloma-induced bone disease, interfere with osteoclast function and prevent osteocyte apoptosis [37]. However, the contribution of the latter to the overall beneficial effects of these drugs on multiple myeloma bone disease remains unknown.

Osteocytes produce molecules that regulate osteoclast production and activity and thus bone resorption. These molecules include the previously mentioned RANKL and macrophage colony stimulating factor 1 (M-CSF), as well as the antiosteoclastogenic cytokine osteoprotegerin (OPG; the RANKL decoy receptor). M-CSF is primarily involved in stimulating the proliferation of osteoclast progenitors, whereas RANKL is essential for their differentiation [38,39]. RANKL is expressed by many different cell types, including osteoblasts and osteocytes, stromal cells, B and T lymphocytes, synovial fibroblasts and hypertrophic chondrocytes [40]. However, only mice lacking RANKL in osteocytes (not osteoblasts or stromal osteoblastic cells) exhibit osteopetrosis, as a result of decreased osteoclast numbers [11,12]. These results indicate that osteocytes are a major source of RANKL and may play a key role in the enhanced resorption displayed in multiple myeloma.

In multiple myeloma, the balance of RANKL and OPG, key determinant for osteoclast differentiation, is disrupted, promoting activation of osteoclasts differentiation and consequently bone destruction [41,42,43²²]. Further, increased levels of soluble RANKL in multiple myeloma patients were shown to be associated with disease activity [44]. Consistent with an important role of this molecule in multiple myeloma bone disease, RANKL neutralization delayed multiple myeloma progression in mice [45]. Further, in patients with multiple myeloma, a monoclonal antibody capable of blocking RANKL (denosumab) reduced bone turnover [46]. However, the specific origin of the elevated RANKL levels in multiple myeloma bone disease is still unclear. RANKL is expressed at both the mRNA and the protein level in multiple myeloma cells themselves purified from bone marrow aspirates of multiple myeloma patients. Further, in-vitro experiments demonstrated that multiple myeloma cells induced differentiation of functional osteoclast precursors [44,47]. In addition, multiple myeloma cells induce RANKL expression in stromal/osteoblastic cells inducing an imbalance of RANKL/OPG ratio in favor of RANKL [48], and multiple myeloma cells also upregulate RANKL in T lymphocytes [49]. These results suggest that several cells can directly contribute to the pool of RANKL in bone. Recent results reported by our laboratory demonstrated for the first time that multiple myeloma cells upregulate RANKL expression in murine osteocytic cells, supporting the idea that osteocytes also contribute to the increased levels of RANKL [50²²]. In addition, we also found that OPG expression is downregulated in osteocytes by multiple myeloma cells,

leading to a more pro-osteoclastogenic RANKL/OPG ratio. In contrast, Giuliani *et al.* [33^{***}] reported that RANKL was not detected in the conditioned medium of the cocultures of human preosteocytes and multiple myeloma cells, suggesting that the contribution of osteocytes to the exacerbated bone resorption induced by multiple myeloma may also depend on other factors released by osteocytes. Further experiments are required to identify the specific role of osteocyte apoptosis and osteocytic RANKL in the early steps of multiple myeloma-induced bone disease.

REGULATION OF BONE FORMATION BY OSTEOCYTES

Osteocyte-derived molecules also regulate the activity of osteoblasts. Only osteocytes in bone secrete sclerostin, the product of the *Sost* gene, a potent inhibitor of bone formation and the first discovered and long-sought molecular mediator between osteocytes and bone surface cells [51]. Sclerostin antagonizes canonical Wnt signaling by binding to Wnt coreceptors LRP-4, LRP-5, and LRP-6, thus inhibiting wnt-dependent osteoblast differentiation and osteoblast survival [10,52–54]. Functional mutations of the *Sost* gene cause abnormal skeletal phenotypes in humans, characterized by high bone mineral density [55,56]. Likewise, *Sost* KO mice have a high bone mass phenotype characterized by increased bone formation [57]. Further, several clinical studies have shown that circulating sclerostin levels increase with age, suggesting that the increased production of sclerostin leads to the impairment of bone formation associated with aging [58]. In multiple myeloma, sclerostin levels are elevated in the serum or the plasma of patients, and correlate with the extent of bone disease and other adverse myeloma features [59,60,61^{**}]. Several recent studies reported that myeloma cells produce sclerostin or induce sclerostin expression in myeloma patients [62,63]; however, the specific contribution of sclerostin derived from osteocytes has not been explored. Giuliani *et al.* [33^{***}] did not find significant differences in sclerostin expression by osteocytes in multiple myeloma patients when compared with healthy controls, suggesting that higher sclerostin levels could be secreted directly by multiple myeloma cells rather than by osteocytes. However, we found that multiple myeloma cells promote upregulation of *Sost* mRNA levels in osteocytes, decreasing Wnt signaling and downregulating Wnt target genes such as OPG [50^{**}]. In contrast, we did not find *Sost* mRNA transcripts in the multiple myeloma cell line JJN3. Furthermore, we cannot exclude the possibility that apoptotic osteocytes in multiple myeloma contribute to the increased sclerostin levels, as it has been reported that proapoptotic factors may induce sclerostin expression in osteocytes [64]. These results suggest the possibility that osteocyte-derived sclerostin contributes to the inhibition of the bone forming activity of osteoblasts in multiple myeloma bone disease. In addition to its role as a potent inhibitor of bone formation, recent findings suggest that sclerostin may also have a catabolic action, promoting osteoclast formation and activity, in a RANKL-dependent manner [65]. Although more experiments are needed to determine the mechanisms leading to the elevated levels of sclerostin in multiple myeloma bone disease and their consequences, Wnt proteins and their inhibitors offer a potential for the development of novel anabolic therapeutics in multiple myeloma.

CONCLUSION

The potential role of osteocytes in multiple myeloma is underexplored. The studies reviewed in this article suggest that osteocytes may be responsible for many of the deleterious effects present in multiple myeloma bone disease (Fig. 2). Osteocytes are an important source of RANKL and sclerostin in bone, and the levels of these molecules are altered in multiple myeloma. The source of these molecules and the mechanisms of their aberrant production, as well as their roles in tumor growth, osteoclastogenesis and bone formation in multiple myeloma bone lesions still remain obscure. Findings demonstrating that multiple myeloma triggers osteocyte apoptosis, followed by increased osteoclast recruitment, bone destruction and poor bone quality, provide a new direction of mechanistic research that has not yet been explored. In addition, the knowledge that multiple myeloma cells regulate sclerostin, as well as Wnt signaling in osteocytes, raises the possibility of using currently available tools that target these pathways as new therapeutic approaches for multiple myeloma, and eventually other cancers targeting bone.

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▪▪ of outstanding interest

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KEY POINTS

- Osteocytes are key regulators of bone remodelling, and their role in multiple myeloma bone disease is largely unknown.
- Osteocyte viability is decreased in multiple myeloma patients with bone lesions.
- Crosstalk between multiple myeloma cells and osteocytes induces profound changes in osteocytic gene expression.
- Understanding multiple myeloma–osteocyte interactions has the potential to develop new therapeutic strategies.

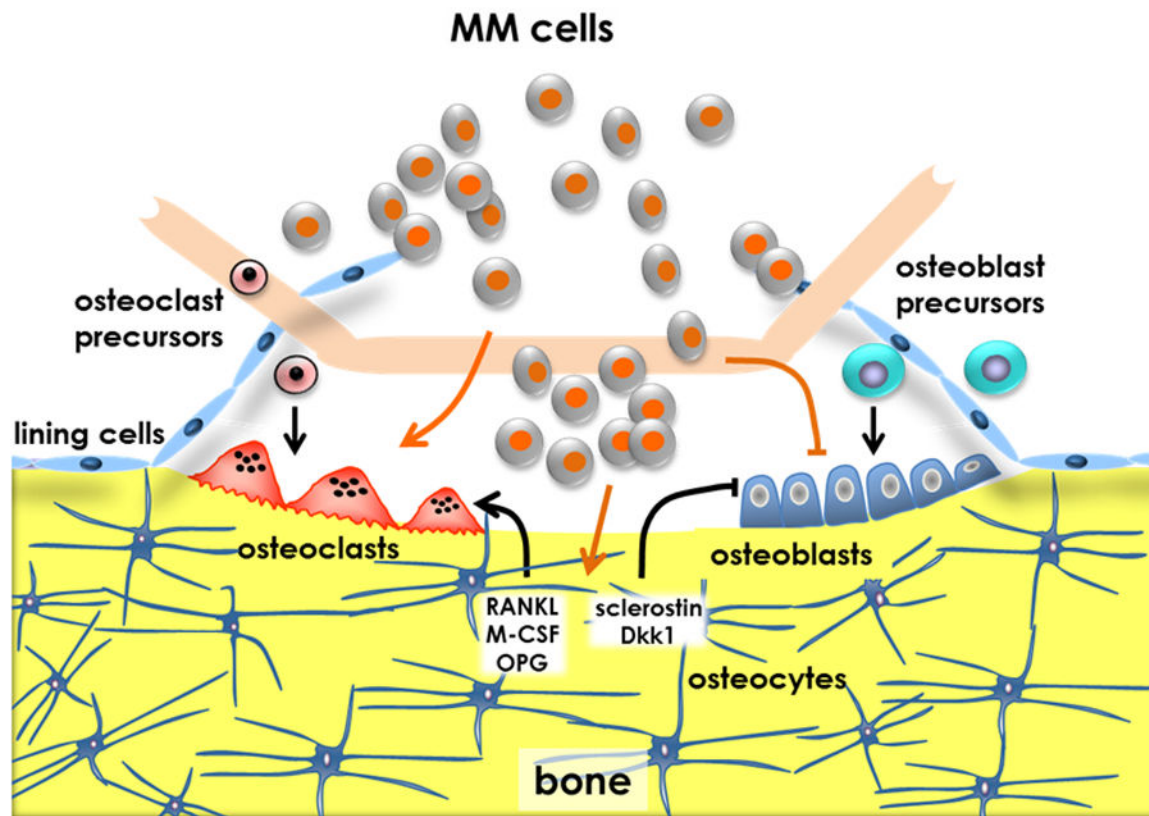


FIGURE 1.

The BRC in multiple myeloma. Osteocytes sense the need for bone resorption and send signals to lining cells, which retract from the bone surface to form the BRC, and secrete molecules that regulate osteoblast and osteoclast formation and activity. Osteoclast precursors are transported to the BRC by marrow capillaries, differentiate to mature osteoclasts and initiate bone remodeling. Osteoblasts precursors from the bone marrow or the circulation differentiate into mature, bone synthesizing cells in response to factors released from the bone matrix by resorption. In multiple myeloma, the canopy of lining cells that separate the bone marrow from the active bone remodeling areas on bone is disrupted. Multiple myeloma cells are then able to enter in close contact with osteoclasts, osteoblasts and osteocytes and modulate their function (dotted lines). BRC, bone remodeling compartment; MM, multiple myeloma; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kappa B ligand.

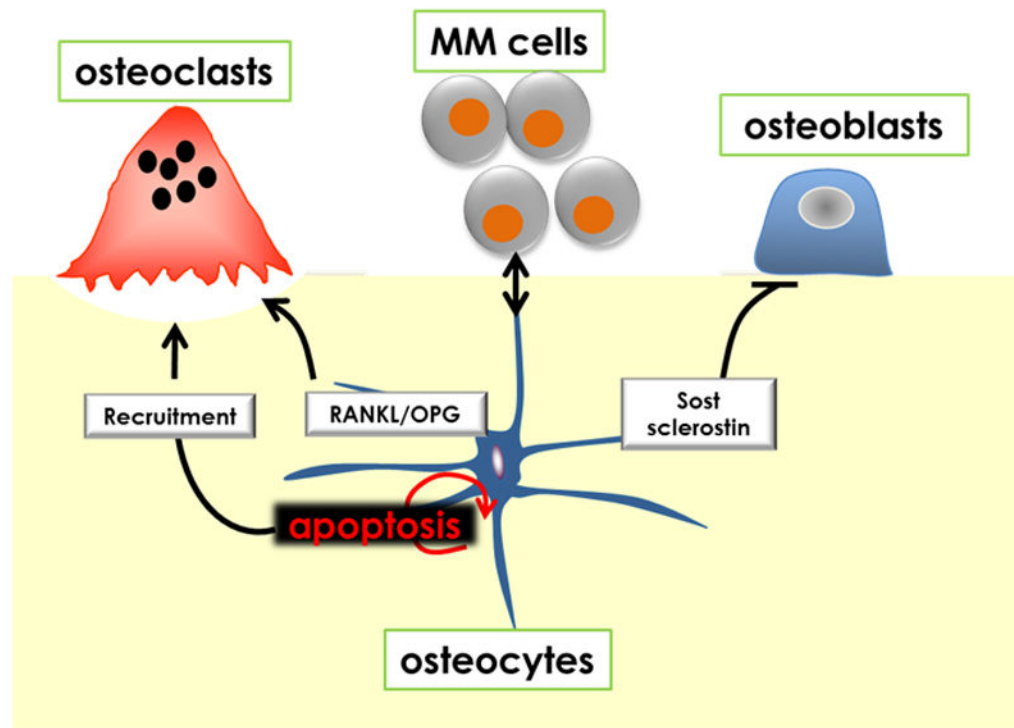


FIGURE 2.

Potential role of osteocytes in multiple myeloma bone disease. Interactions between osteocytes and multiple myeloma cells within the BRC modulate critical signaling pathways and mediators responsible for bone resorption and bone formation. Osteocyte apoptosis is markedly increased in multiple myeloma patients with bone lesions and results in targeted osteoclast recruitment. The RANKL/OPG ratio in osteocytes is also elevated by multiple myeloma cells because of a combination of downregulation of OPG and upregulation of RANKL expression potentially increasing osteoclast differentiation. In addition, increased Sost/Sclerostin in osteocytes induced by multiple myeloma could contribute to the decreased bone formation that characterizes multiple myeloma-induced bone disease. MM, multiple myeloma; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kappa B ligand.